

## Survival of *Campylobacter* spp. on inoculated pork skin or meat.

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### Abstract

*Campylobacter* is one of the main causes of human foodborne bacterial zoonoses due to food consumption in developed countries. Nine to 32% of pig carcasses are contaminated by *Campylobacter*. The purpose of the study was to improve our knowledge of the survival of implanted campylobacters from the two kinds of pork matrix meat (skin, muscle) during meat cold domestic storage. One hundred and twenty pork skin and 120 skinless chine samples (25 cm<sup>2</sup>/sample) were inoculated with two *C. jejuni* and four *C. coli* strains and stored in closed box at 4 °C for 1, 4, 8, 15 and 22 days. *Campylobacter* were isolated from sample suspensions after mechanical pummeling and numbered by direct plating. We calculated the shoulder time (ST), the D value (the time for one log decrease) and the R<sub>1</sub> value (the time to reach 10% of the initial population  $R_1 = ST + D$ ). We compared them in a stratified approach according to pork matrix and strain. According to matrixes, mean D, TS and R<sub>1</sub> value varied significantly between pork skin (4.3 days, 1.3 days, 5.6 days, respectively) and spare rib (7.2 days, 3.5 days, 10.8 days, respectively). On spare rib, R<sub>1</sub> was higher (16 days) with one *C. coli* strain (CCV55). Statistical effects between TS and R<sub>1</sub> value on spare rib and strain were noticed. This study shows that the survival of campylobacters on pork meat is similar to the survival of *Campylobacter* on poultry meat. Consequently, good hygiene practices are needed to manage the risk of pork *Campylobacter* contamination and further studies focusing on survival factors may complete this risk analysis on the pork food chain.

### Introduction

*Campylobacter jejuni* and *C. coli* are responsible for the main foodborne bacterial zoonoses in developed countries (OMS 2000). Only around one hundred bacteria are needed to induce abdominal pain or gastro-enteritis and even Guillain – Barré (ROBINSON et al. 1979, BLACK et al. 1988). The prevalence of meat contamination by thermophilic *Campylobacter* has been reported to reach 90% for poultry meat and 60% for red meat (pork, bovin). Pork is the most consumed meat in the European Union (DEVINE, 2003). In pork primary production, *Campylobacter coli* carriage is high (PAYOT et al. 2004, PEARCE et al. 2003, HARVEY et al. 1999, WIETJENS et al. 1999) and many studies have reported that from 9 to 32% of pig carcasses are contaminated. Even if slaughterhouse hygiene is a determining factor for managing pig carcass contamination (MAGRAS et al. 2006), little information is available about the survival of *Campylobacter* on pork during meat cold domestic storage.

Previous studies performed on chicken (LEE et al. 1998, SOLOW et al. 2003, YOON et al. 2004) and pork (SOLOW et al. 2003, FOSSE et al. 2006) have shown a protective effect of the matrix (skin versus muscle). But among all the factors affecting campylobacters survival, two other factors can be quoted: i) endogen flora level which can compete with campylobacters, ii) a meat matrix / *Campylobacter* spp. strain competition. Furthermore excepted Yoon et al. (2004), studies on survival of *Campylobacter* described only the survivor curve without fitting the data to a linear model. To our knowledge, such a



definition of *Campylobacter* survival on the two kinds of pork matrix (skin and meat) has not been carried out to date. This kind of data must be taken into account to apply risk analysis for food safety.

The purpose of the study was to improve our knowledge of the survival of different implanted *Campylobacter* strains on retailed pork skin and meat during the meat cold domestic storage.

## Material and methods

***Campylobacter* strains.** Six strains of *Campylobacter* were studied: *C. jejuni* NCTC 11168, a sequenced human feces strain (28); wild *C. jejuni* isolated in human campylobacteriosis (CjBOF); *C. coli* CIP 70.81, a pig feces strain; three *C. coli* wild strains (CcV055, CcV639 et CcV782) isolated from pig carcasses in a slaughterhouse (Magras et al. 2006).

***Pork meat samples.*** Two meat matrices were tested: skinless chine (*trapezius* muscle, *serratus ventralis cervicis* muscle, *andsemispinalis capitis* muscle) and pork skin. The cooled retail meats were purchased from a local butcher, and 5- by 5-cm pieces were excised as samples. Samples of both pork skin (120 samples) and chine (120 samples) were the same thickness, i.e., 0.5 cm. The homogeneity of the thickness of samples was controlled randomly.

**Experimental inoculation of meat samples.** A calibrated quantity of *Campylobacter* colonies from a 48-h culture on Karmali plates (AES Laboratoires, Combourg, France) was inoculated into 20 mL of BHI broth (Oxoid, Dardilly, France). After 24 h of incubation under microaerophilic conditions, 1 mL of this liquid culture was inoculated into 100 mL of BHI and incubated for 48h under microaerophilic conditions to obtain the parent culture. To assess the absence of bacterial contamination of parent cultures, 0.1 mL of the culture was streaked onto Karmali and PCA plates and then cultured. The surfaces of the meat samples (5- by 5-cm piece of chine or pork skin) were inoculated with 0.1 mL of parent culture. After inoculation, samples were stored for 1, 4, 8, 15 and 22 days in hermetically sealed boxes at 4°C. In each series of four analyses, the sample clusters contained inoculated samples and noninoculated samples.

**Bacterial analysis.** Recovery method for separating *Campylobacter* from meat samples and obtaining a bacterial suspension was mechanical homogenization (pummeling with 10 mL of sterile peptone water for 60 seconds in a stomacher bag with a filter). A 0.1 mL volume of two dilutions of bacterial suspensions obtained was streaked on two Karmali with a spiral plater (Eddyjet, IUL SA, Barcelona, Spain) and incubated under microaerophilic conditions at 42°C. After 72 h of incubation, colonies on plates were enumerated.

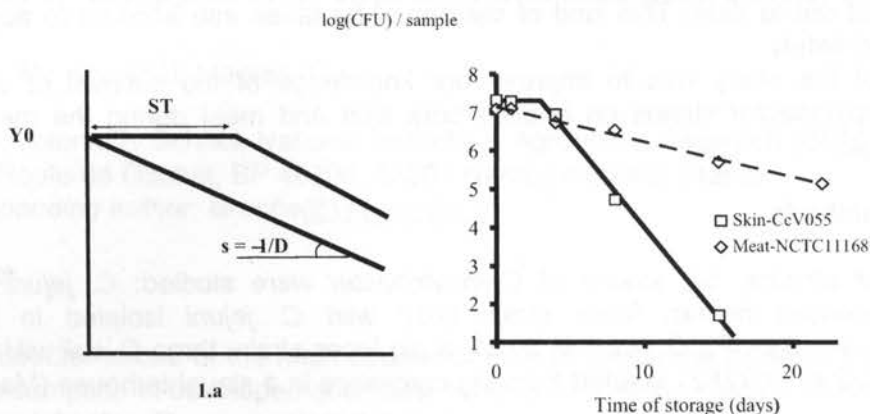
**Survivor curves and parameters calculation:** Each survivor curve was generated by fitting the data (5 samples/curve, 1 sample/time) to the linear model developed by Buchanan et al. (1993) (figure 1).

$$Y = Y_0 + s(t - ST)$$

$Y$  = log count of bacteria at time  $t$  (log (CFU/sample));  $Y_0$  = log count of bacteria at time  $t = 0$  represents the number of *Campylobacter* inoculated on the sample (log (CFU/sample));  $s$  = slope of the survivor curve (log (CFU/sample)/day);  $t$  = time (days);  $ST$  = duration of lag period prior to initiation of inactivation or the shoulder time (days).

The  $D$  values were then calculated by taking the negative reciprocal of  $s$ . The time (days) to a 1- $D$  (the time for one log decrease) inactivation ( $R_1$ ) was calculated using the equation:

$$R_1 = ST + D$$



**Figure 1:** 1.a The linear model of survivor curve of Buchanan et al. (1993) used to calculate the 3 parameters ( $ST$ ,  $D$ ,  $R_1$ ); 1-b example of one of the 24 survivor curves of *Campylobacter* sp. on pork meat and of the 24 survivor curves on pork skin generated.

**Statistical analysis.** Data were analyzed with SAS software (SAS Institute, Cary, North Carolina) using a general linear model (PROC GLM) which is a sum of squares difference analysis of variance. We compared them in a stratified approach according to pork matrix and strain.

## Results

Twenty four survivor curves of *Campylobacter* on skinless chine (meat) and 24 survivor curves of *Campylobacter* on pork skin were generated. According to matrices, mean D, TS and  $R_1$  value varied significantly (table 1) between pork skin: 4.3 days, 1.3 days, 5.6 days, respectively and pork meat: 7.2 days, 3.5 days, 10.8 days, respectively.

**Table 1:** Mean values of *Campylobacter* survivor parameters (D, ST,  $R_1$ ) according to pork matrices.

		D (days)	ST (days)	$R_1$ (days)
Pork skin	n	24	24	24
	mean value	4.3	1.3	5.6
	$\sigma$	2.0	2.2	2.9
	Minimal value	2.1	0	2.3
	Maximal value	10.5	7.5	11.8
Pork meat (skinless chine)	n	24	24	24
	mean value	7.2	3.5	10.8
	$\sigma$	3.4	4.6	4.4
	Minimal value	2.6	0	3.7
	Maximal value	16.4	15.1	18.6
p value of matrix effect $Pr > F$		0.001	0.04	<.0001

n: number of survivor curves generated; D: negative reciprocal of slope of the survivor curve; ST: duration of lag period prior to initiation of inactivation or the shoulder time;  $R_1$  the time for one log decrease = ST + D

On pork meat,  $R_1$  varied from 8.0 to 16.1 days for the different strains. On pork skin,  $R_1$  for the different *Campylobacter* strains are not statistically different (table 2).

**Table 2:** Comparisons of adjusted mean  $R_1$  obtained on pork skin and pork meat for the different *Campylobacter* strains.

Strain	Pork skin			Pork meat		
	n	$R_1$	Pr>F	n	$R_1$	Pr>F
CCV055	4	7.6	NS	4	16.1	a 0.013
CCV639	4	5.5	NS	4	8.6	b 0.18
CCV782	4	4.7	NS	4	11.3	ab 0.07
CIP7081	4	4.2	NS	4	8.7	b 0.38
CJBOF	4	6.7	NS	4	8.0	b 0.62
NCTC11168	4	4.2	NS	4	10.3	b 0.007

a, b : statistical difference with  $\alpha = 5\%$ , NS: no statistical difference with  $\alpha = 5\%$ .

## Discussion

We confirm the high survivability of *Campylobacter* on pork meat during cold domestic storage conditions (SOLOW et al. 2003). Furthermore this survivability of *Campylobacter* on pork meat appears similar to the survivability of *Campylobacter jejuni* on poultry meat (with mean ST 7 days, D 4 to 5 days, YOON et al. 2006). However our study shew variations of the three survivor parameters (D, ST,  $R_1$ ) in function of pork matrix, since parameters obtained from pork meat were significantly higher than parameters obtained from pork skin. The less survivability of *Campylobacter* on pork skin could be explained by skin nature (malpighian epithelium). This tissue has less directly available nutriments,



which can stress trophical competition. Mean mesophile flora contamination levels were not different on pork skin and pork meat (results not shown). Endogenous flora can not explain differences between those two kinds of pork matrix.

## Conclusion

Data of the present study confirm that the survivability of *Campylobacter* sp. on pork matrix (skin and meat) in cold domestic storage conditions is similar to the survivability of *Campylobacter jejuni* on poultry meat. Consequently, good hygiene practices are needed to manage the risk of pork contamination by *Campylobacter* and further studies focusing on survival factors may complete this risk analysis on the pork food chain.

## Acknowledgements

The authors thank A. Rossero, F. Jugiau and F. Rama for their technical assistance.

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